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Effects of Low-Protein Diets Supplemented with Essential Amino Acids on Growth Performance, Meat Quality, and Nitrogen Retention in Growing Japanese Quails

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Abstract
One experiment was carried out to investigate the effect of low crude protein (CP) diets supplemented with crystalline essential amino acids (EAAs) on growth performance, meat quality, and nitrogen retention in Japanese quails reared from 1 to 35 days of age. A total of 840 unsexed one-day-old quail chicks were used in a completely randomized design consisting of seven treatments with six replicates and 20 birds in each. In addition to the control diet, two negative control diets were also adjusted to contain 10 and 20% lower CP than the control (LP10 and LP20 diets, respectively). The EAAs/CP ratio was similar to that of the control diet. Also, four rations supplemented with amino acids were formulated to contain 10% (LP10+EAAs10 and LP20+EAAs10) or 20% (LP10+EAAs20 and LP20+EAAs20) higher EAAs than LP10 and LP20. Crude protein digestibility was determined using the total collection method at 21 and 35 d of age. After slaughtering at 21 and 35 d of age, whole-breast meats were dissected out to determine meat quality indices. During 1 - 21d of age, 10 or 20% reduction in CP significantly decreased body weight gain and increased feed conversion ratio; however, amino acid supplementation significantly improved body weight gain and feed conversion ratio ($P < 0.05$). During 1- 35 d of age, reduction of CP to 20% led to decreased body weight gain in EAAs supplemented and un-supplemented diets. Dietary treatments did not have any significant effect on the breast meat quality variables except for pH. At 35d of age, 20% reduction in dietary CP significantly reduced nitrogen retention percentage. Overall, a 10% reduction in dietary CP without EAAs supplementation had no adverse effect on the growth performance of Japanese quails during the 1 to 35d of age.

Introduction
Protein is a costly ingredient in poultry diets. On the one hand, insufficient levels of amino acids can lead to lower performance, because deficiency of amino acids restricts protein synthesis (Lima *et al.*, 2016). On the other hand, the over-consumption of protein is not economical since it leads to the degradation of surplus amino acids (Perry *et al.*, 2004). Poultry excreta and its nitrogenous compounds resulting in nitrite or nitrate contamination of water, and subsequently increases air pollution due to the release of ammonia (Vieira and Angel, 2012). Therefore, one of the most important strategies to reduce environmental pollution is to reduce nitrogen excretion in poultry manure. Partial replacement of dietary protein during the growing and laying phases by appropriate amino acids has been the issue of many experiments in recent decades (Si *et al.*, 2004; Abudabos and Aljumaah, 2012; Belloir *et al.*, 2017;

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Saraiva *et al*., 2020). However, there are restrictions on how much intact protein can be substituted by free amino acids without affecting growth performance (Si et *al*., 2004; Baker, 2009; Liu *et al*., 2021).

The CP content in the diet of growing quails ranges from 22 to 27% in the different reports (NRC, 1994; Baldini *et al*., 1995; Shrivastava and Panda, 1999; Rostagno *et al*., 2011). In most of these investigations, just one diet has been suggested for all of the growing phases. Besides, it is uncertain that protein recommendations by NRC (1994) as a basic reference are appropriate for modern quail strains.

It seems that more accurate estimates of amino acid requirements in quails would allow to partially replace dietary CP with industrial amino acids in crystalline form. This replacement could improve the amino acid balance, avoid excess oxidation, reduce metabolic costs and emission of pollutants into the environment and unfavorable aroma in aviculture (Lemme, 2003; Ayasan *et al*., 2009; Vieira and Angel, 2012). Ayasan and Okan (2010) and Wen *et al*. (2017) suggested that with supplementation of synthetic amino acids, it is feasible to formulate low-protein diets without any detrimental effect on performance and carcass traits in broilers and quails. Taheri and Alvani (2020) showed that lower dietary protein concentration without any reduction in EAAs concentration reduced mortality and improved European Poultry Efficiency Factor without any negative effects on feed conversion ratio, weight gain and carcass characteristics of broilers.

Nevertheless, data on the CP and EAAs requirements of quails during different growing phases are very limited. Therefore, in the present study, we examined the effects of low CP diets supplemented with essential amino acids on growth performance, carcass characteristics, meat quality and nitrogen retention in Japanese quail.

Materials and Methods

Bird management and experimental design

All procedures were confirmed by the Institutional Animal Care and Use Committee of the Shahid Bahonar University of Kerman (approval number: IR.UK.VETMED.REC.1400.008). A total of 840 unsexed one-day-old Japanese quails were used in a completely randomized experimental design, distributed into seven treatments with six replicates of 20 birds each and reared for 35 days. The average initial weight of quails was 9.22 ± 0.21g. Chicks were placed in wire cages with dimensions of 100 × 50 cm. The mean initial body weight was similar among all pens. Throughout the experiment, chicks were reared under a 24h lighting program and standard temperature regimen, 38°C during 1 to 2 d of age and gradually decreased until reaching 25°C at 23d of age.

Before diet formulation, the feed ingredients were analyzed using a near-infrared instrument at Degussa Evonik, Iran. The experiment was conducted using a 2-phase feeding program (1–21 and 22–35 d). The diets were isoenergetic, with the CP and EAAs (including lysine, methionine, cysteine, threonine, and isoleucine) contents of the control diet formulated according to Silva and Costa (2009). Two negative control diets were formulated to contain 10 and 20% lower CP than the control diet (LP10 and LP20 diets, respectively), but EAAs was held at a constant ratio to CP for obtaining the same ratio in the two diets. Also, four amino acid-supplemented diets were formulated to supply 10% (LP10+EAAs10 and LP20+EAAs10) or 20% (LP10+EAAs20 and LP20+EAAs20) higher EAAs than LP10 and LP20 diets (Tables 1 and 2). Feed and water were available *ad-libitum* during the study.

Performance and carcass characteristics

The body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) were recorded weekly. At 21 and 35 d of age, two quails (one male and one female) from each replication were randomly selected and weighed individually before slaughtering. The quails were deprived of feed for four hours and then slaughtered by severing the jugular vein. The quails were skinned, and wingtips eliminated. The weight of the major parts, including the thighs, breast abdominal fat, liver, heart, gizzard, proventriculus, and intestine, were recorded and expressed as a percentage of the live body weight (BW).

Meat quality

After slaughtering the birds at 21 and 35 d of age, whole breast meat from two quails (one male and one female) per replicate was dissected out and stored in nylon bags at 4°C for meat quality analysis (i.e., pH, cooking loss, dripping loss, and water holding capacity).

The pH of the breast meat was measured according to Jang *et al.* (2008). Five grams of raw breast meat was homogenized with 25 mL of distilled water and then filtered. Finally, the pH was measured with a pH meter (knick-766 laboratory pH meter) at room temperature (22±2°C).

For measuring water holding capacity (WHC), 1 g of the meats was placed on a tissue paper inside a tube and centrifuged for 4 min at 1500 × g. The samples were then dehydrated at 70 °C for 24 h. WHC was calculated according to this formula: [(weight after centrifugation−weight after drying)/ initial weight] × 100 (Castellini *et al.*, 2002).

Table 1. Ingredients and nutrient composition of the experimental diets (1 to 21 d, as-fed basis).

Ingredient (%)	Treatments[1]						
	Control	LP10	LP20	LP10+ EAAs10	LP20+ EAAs10	LP10+ EAAs20	LP20+ EAAs20
Corn (CP=7.78%)	51.57	55.61	57.70	55.37	57.74	55.14	60.45
Soybean meal (CP=43.60%)	33.21	33.86	32.32	33.81	32.08	33.75	29.47
Corn gluten meal (CP=75.87%)	7.17	3.37	0.48	2.93	0.19	2.46	1.05
Wheat bran (CP=14.23%)	4.50	3.49	5.21	3.75	5.38	4.03	4.83
Soybean oil	0.50	0.80	1.50	0.89	1.50	0.96	0.62
Dicalcium phosphate	0.98	0.99	1.01	0.99	1.01	0.99	1.03
Calcium carbonate	0.76	0.75	0.75	0.75	0.75	0.75	0.75
NaCl	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Vitamin premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[3]	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Met 99 (%)	0.10	0.09	0.09	0.18	0.16	0.26	0.25
L-Lys HCl 78.5 (%)	0.25	0.11	0.03	0.26	0.16	0.39	0.34
L-Thr 98 (%)	0.11	0.08	0.06	0.17	0.14	0.26	0.23
L-Ile 98 (%)	0	0	0	0.05	0.04	0.15	0.13
L-Val 98(%)	0	0	0	0	0	0.01	0
Composition							
AME_n(kcal/kg)	2900	2900	2900	2900	2900	2900	2900
CP (%)	25	22.5	20	22.5	20	22.5	20
Digestible Lys (%)	1.19	1.07	0.95	1.18	1.05	1.28	1.14
Digestible Met (%)	0.47	0.42	0.37	0.49	0.43	0.57	0.52
Digestible Met+Cys (%)	0.80	0.72	0.64	0.79	0.70	0.86	0.79
Digestible Thr (%)	0.87	0.78	0.69	0.86	0.76	0.74	0.83
Digestible Ile (%)	0.92	0.83	0.74	0.88	0.78	0.96	0.85
Digestible val (%)	0.99	0.91	0.85	0.89	0.84	0.89	0.82
Calcium (%)	0.60	0.60	0.60	0.60	0.60	0.60	0.60
Available phosphorus (%)	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Chloride (%)	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Potassium (%)	0.92	0.93	0.91	0.90	0.90	0.92	0.86
Sodium (%)	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Diet cost: Rials/kg of diet	43380	42120	39840	82890	73530	256800	221820
Composition analysis[4]							
CP (%)	25.2	22.4	20.2	22.8	19.8	22.6	20.15
Digestible Lys (%)	1.21	1.08	0.93	1.20	1.06	1.29	1.16
Digestible Met (%)	0.46	0.43	0.36	0.50	0.44	0.56	0.53
Digestible Met+Cys (%)	0.78	0.74	0.65	0.80	0.70	0.88	0.78
Digestible Thr (%)	0.87	0.77	0.68	0.87	0.78	0.95	0.85
Digestible Ile (%)	0.93	0.82	0.75	0.90	0.79	0.97	0.87
Digestible Arg (%)	1.34	1.30	1.21	1.30	1.20	1.30	1.14
Digestible Trp (%)	0.23	0.21	0.21	0.22	0.21	0.22	0.20
Digestible Leu (%)	2.26	1.87	1.53	1.82	1.49	1.77	1.54
Digestible Val (%)	1.02	0.93	0.84	0.91	0.83	0.91	0.81
Digestible Phe (%)	1.16	1.02	0.89	1.00	0.87	0.98	0.86
Digestible His (%)	0.56	0.53	0.49	0.52	0.48	0.52	0.47

[1]Control, control diet, LP10 & LP20, containing 10 and 20% lower CP than control diet, respectively, LP10+ EAAs10 & LP20+EAAs10, containing 10% higher EAAs than LP10 & LP20, LP10+EAAs20 & LP20+EAAs20, containing 20% higher EAAs than LP10 & LP20.
[2]Vitamin premix provided the followings per kilogram of diet: vitamin A, 12000 IU, cholecalciferol, 5000 IU, vitamin E, 45 IU, vitamin K_3, 2.4 mg, thiamine, 2.6 mg, riboflavin, 6.6 mg, pantothenic acid, 25 mg, niacin, 55 mg, choline chloride, 500 mg, biotin, 0.1 mg, folic acid, 1.5 mg, pyridoxine 5.5 mg, vitamin B_{12},0.015 mg, BHT, 1 mg,
[3]Mineral premix provide the followings per kilogram of diet: iron, 50 mg, zinc, 85 mg, manganese, 90 mg, iodine, 1 mg, copper, 10 mg, selenium, 0.25 mg. [4]Amino acid analysis was done by Degussa Evonik (Iran).

For cooking loss determination, meat samples of 1 cm^3 were cut from each breast muscle and weighed (W_1). Then, the samples were cooked at 85 °C for 10 min and reweighed (W_2). The following equation was used to calculate cooking loss: Cooking loss = 100 × [(W_1− W_2)/ W_1] (Bertrama *et al.*, 2003).

For estimation of the dripping loss, each breast meat sample was weighed and put into a cotton bag, and transferred into a plastic bag, which was completely sealed and kept in a refrigerator at 4 °C for 24 h. After 24 h, the meat sample was reweighted. The dripping loss was computed as the percentage of weight loss over the initial sample weight (Christensen, 2003).

Table 2. Ingredients and nutrient composition of the experimental diets (22 to 35 d, as-fed basis).

Ingredient (%)	Treatments[1]						
	Control	LP10	LP20	LP10+EAAs10	LP20+EAAs10	LP10+EAAs20	LP20+EAAs20
Corn (CP=7.78%)	56.74	62.22	69.70	62.80	70.23	62.12	69.07
Soybean meal (CP=43.60%)	36.05	32.43	26.09	31.71	25.43	29.82	25.18
Corn gluten meal(CP=75.87%)	1.93	0.60	0.60	0.60	0.60	1.35	0.55
Soybean oil	2.74	2.23	1.02	2.06	0.87	2.28	1.27
Dicalcium phosphate	0.73	0.77	0.83	0.78	0.83	0.80	0.84
Calcium carbonate	.63	0.64	0.65	0.64	0.65	0.64	0.64
Filler(sand)	0	0	0	0	0	1.2	0.80
NaCl	0.36	0.36	0.36	0.36	0.36	0.36	0.36
Vitamin premix[2]	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Mineral premix[3]	0.25	0.25	0.25	0.25	0.25	0.25	0.25
DL-Met 99 (%)	0.14	0.11	0.08	0.19	0.15	0.25	0.20
L-Lys HCl 78.5 (%)	0.05	0.03	0.07	0.17	0.20	0.33	0.29
L-Thr 98 (%)	0.13	0.11	0.1	0.19	0.17	0.28	0.24
L-Ile 98 (%)	0	0	0	0	0.01	0.07	0.06
Composition							
AME_n(kcal/kg)	3050	3050	3050	3050	3050	3050	3050
CP (%)	22	19.8	17.6	19.8	17.6	19.8	17.6
Digestible Lys (%)	1.05	0.94	0.84	1.04	0.92	1.12	0.99
Digestible Met (%)	0.45	0.39	0.34	0.46	0.40	0.53	0.45
DigestibleMet+Cys (%)	0.74	0.66	0.59	0.73	0.65	0.79	0.69
Digestible Thr (%)	0.82	0.74	0.65	0.81	0.72	0.87	0.77
Digestible Ile (%)	0.83	0.74	0.65	0.73	0.65	0.79	0.69
Digestible Val (%)	0.94	0.81	0.70	0.81	0.70	0.78	0.74
Calcium (%)	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Available phosphorus (%)	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Chloride (%)	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Potassium (%)	0.92	0.89	0.92	0.90	0.89	0.92	0.86
Sodium (%)	0.14	0.14	0.14	0.14	0.14	0.14	0.14
Diet cost: Rials/kg of diet	41700	39510	37440	40050	46560	139920	125490
Composition analysis[4]							
CP (%)	22.15	19.6	17.7	19.75	17.6	20.0	17.75
Digestible Lys (%)	1.03	0.93	0.82	1.06	0.95	1.13	1.01
Digestible Met (%)	0.47	0.39	0.33	0.47	0.41	0.55	0.46
Digestible Met+Cys (%)	0.75	0.67	0.58	0.71	0.67	0.80	0.71
Digestible Thr (%)	0.84	0.75	0.64	0.82	0.74	0.88	0.78
Digestible Ile (%)	0.83	0.75	0.66	0.72	0.66	0.80	0.68
Digestible Arg (%)	1.30	1.19	1.02	1.17	1.00	1.12	0.99
Digestible Trp (%)	0.23	0.21	0.17	0.20	0.17	0.19	0.17
Digestible Leu (%)	1.76	1.55	1.43	1.54	1.42	1.56	1.39
Digestible Val (%)	0.92	0.83	0.74	0.82	0.73	0.81	0.72
Digestible Phe (%)	0.99	0.89	0.79	0.87	0.77	0.87	0.76
Digestible His (%)	0.53	0.48	0.44	0.48	0.43	0.47	0.42

[1]Control, control diet, LP10 & LP20, containing 10 and 20% lower CP than control diet, respectively, LP10+EAAs10 & LP20+EAAs10, containing 10% higher EAAs than LP10 & LP20, LP10+EAAs20 & LP20+EAAs20, containing 20% higher EAAs than LP10 & LP20
[2]Vitamin premix provided the followings per kilogram of diet: vitamin A, 12000 IU, cholecalciferol, 5000 IU, vitamin E, 45 IU, vitamin K_3, 2.4 mg, thiamine, 2.6 mg, riboflavin, 6.6 mg, pantothenic acid, 25 mg, niacin, 55 mg, choline chloride, 500 mg, biotin, 0.1 mg, folic acid, 1.5 mg, pyridoxine 5.5 mg, vitamin B_{12},0.015 mg, BHT, 1 mg,
[3]Mineral premix provide the followings per kilogram of diet: iron, 50 mg, zinc, 85 mg, manganese, 90 mg, iodine, 1 mg, copper, 10 mg, selenium, 0.25 mg. [4]Amino acid analysis was done by Degussa Evonik (Iran).

Digestible Nitogen determination

Digestibility of CP was determined using the total collection method at the end of third and fifth weeks of age. Briefly, excreta were collected, and the related FI was recorded for the subsequent three days. The excreta were collected daily and frozen until drying at the end of the experiment. Total nitrogen and dry matter of dried samples of the excreta and diets were determined.

Statistical analysis

Data were subjected to a one-way analysis of variance using the GLM procedure of SAS (SAS.,

2010) as a completely randomized design. For performance traits, the pen was the experimental unit, whereas individual bird data were used for the weight of internal organs. The means were compared using the Duncan's Multiple Range Test.

Results

Growth performance

Data for growth performance are presented in Table 3. During 1-21 d of age, the reduction of CP significantly decreased BWG and impaired FCR ($P < 0.01$). No differences were found in growth performance of the birds fed with control, LP10+EAAs10 and LP10+EAAs20 diets. Supplementation of the LP20 diet with 20% higher EAAs, significantly improved BWG and FCR, in comparison to the LP20 and LP20+EAAs10 ($P \leq 0.0001$), but were significantly less than control diet. Also, 20% EAAs supplementation of the LP10 diet significantly improved FCR in comparison to the LP10 diet ($P \leq 0.0001$). Different treatments had no significant effect on feed intake, but feed intake was numerically the highest, for birds fed with LP10+EAAs10 diet. During 22 - 35 d of age, quails fed LP20 diet showed numerically higher BWG and FI than those fed other diets ($P > 0.05$). Also, FCR in the birds fed LP10, LP20, and LP20+EAAs10 diets were significantly better than the control ($P < 0.01$).

During the total rearing period (1-35 d), a 20% reduction of CP significantly decreased BWG in comparison to the control, LP10 +EAAs10 and LP10+EAAs20 groups ($P < 0.01$). Also, adding EAAs did not affect the LP20 group, but 20% EAAs supplementation of the LP10 diet significantly improved BWG in comparison to the LP10 diet ($P < 0.01$). Nevertheless, in the whole of the experiment, no differences were observed in FI and FCR between different dietary treatments. Furthermore, different dietary treatments did not affect livability at 21 and 35 d of age ($P > 0.05$).

In the total of the rearing period, enrichment of the diets with EAAs strongly increased feed cost per kg of live body gain, because some EAAs are very expensive.

Table 3. Growth performance of quails fed experimental diets from 1 to 35 d of age.

Variables	Treatments[1]							*P*-value	*SEM*[2]
	Control	LP10	LP20	LP10+ EAAs10	LP20+ EAAs10	LP10+ EAAs20	LP20+ EAAs20		
BWG (g/bird/d)									
1-21 d	5.85^{a}	5.46^{bc}	5.31^{c}	5.83^{a}	5.43^{c}	5.91^{a}	5.61^{b}	0.0001	0.05
22-35 d	7.41	7.53	7.78	7.34	7.51	7.39	7.42	0.064	0.09
1-35 d	6.44^{ab}	6.34^{bc}	6.29^{c}	6.43^{ab}	6.26^{c}	6.52^{a}	6.29^{c}	0.0018	0.04
FI (g/bird/d)									
1-21 d	12.47	12.34	12.45	12.85	12.72	12.77	12.68	0.238	0.14
22-35 d	24.78	24.54	24.87	24.79	24.45	25.57	24.41	0.158	0.28
1-35 d	17.54	17.22	17.47	17.50	17.33	17.94	17.28	0.084	0.15
FCR									
1-21 d	2.15^{d}	2.26^{b}	2.33^{a}	2.21^{bc}	2.33^{a}	2.17^{dc}	2.26^{b}	0.0001	0.01
22-35 d	3.39^{ab}	3.25^{c}	3.22^{c}	3.37^{ab}	3.25^{c}	3.41^{a}	3.28^{bc}	0.0031	0.03
1-35 d	2.72	2.73	2.75	2.72	2.76	2.75	2.73	0.378	0.02
Livability (%)									
21 d	99.16	100.00	98.33	100.00	99.16	100.00	97.5	0.143	0.73
35 d	95.00	95.00	96.66	98.33	96.66	97.50	95.00	0.588	1.52
Feed cost: Rials/kg of BWG									
1-35 d	113180^{c}	111130^{c}	106390^{c}	161580^{b}	162430^{b}	522830^{a}	460620^{a}	0.0001	205.2

[1]Control, control diet, LP10 & LP20, containing 10 and 20% lower CP than control diet, respectively, LP10+EAAs100 & LP20+EAAs10, containing 10% higher EAAs than LP10 & LP20, LP10+EAAs20 & LP20+EAAs20, containing 20% higher EAAs than LP10 & LP20.
Means with different superscripts within the same row differ significantly ($P < 0.05$). [2]Standard error of means.

Carcass traits

The carcass characteristics results are depicted in Table 4. At 21 d of age, 10% amino acid supplementation of the LP10 diet significantly increased breast yield in comparison to the control, LP10, and LP20 diets ($P < 0.05$). The highest liver yield was also obtained with quails fed LP10 diet ($P < 0.05$). Quails fed LP20+EAAs10 diet had numerically higher abdominal fat yield ($P > 0.05$). At 35 d of age, dietary treatments had no significant effect on liver and breast yield ($P > 0.05$). Also, the relative weight of the other parts, including thighs, proventriculus, gizzard, small intestine, heart, and abdominal fat of the birds at both of the growing periods were not affected by the dietary treatments ($P > 0.05$).

Meat quality

Breast meat quality parameters for different groups are presented in Table 5. At 35 d of age, the meat pH of the birds fed with the control diet was significantly lower than the other treatments ($P \leq 0.0001$). Other parameters (cooking loss, dripping loss, and water holding capacity) were not affected by the dietary treatments at both periods.

Nitrogen retention

The effect of different dietary treatments on N balance is illustrated in Table 6. Different treatments affected N balance data. At 21 d of age, quails which fed 20% CP diets, with and without EAAs supplementation (LP20, LP20+EAAs10, and LP20+EAAs20), had significantly lower N intake and N retention compared to those fed with the other diets (*P* < 0.01). N excretion was the lowest in quails fed LP10 and LP20 diets and was significantly different from control and LP10+EAAs20 (*P* < 0.01).

Table 4. Effects of different treatments on the relative weight of different carcass traits (% of live body weight) at 21 and 35 d of age.

Variables		Treatments[1]							*P*-value	*SEM*[2]
		Control	LP10	LP20	LP10+ EAAs10	LP20+ EAAs10	LP10+ EAAs20	LP20+ EAAs20		
Carcass	21 d	56.7	56.5	56.2	57.2	56.7	56.8	57.5	0.476	0.74
	35 d	65.0	63.9	64.5	64.7	64.3	64.6	64.9	0.725	0.81
Breast	21 d	21.5[bc]	21.5[bc]	21.1[c]	22.9[a]	21.8[abc]	22.6[ab]	21.9[abc]	0.0164	0.52
	35 d	25.7	24.9	25.7	26.3	25.9	26.6	25.8	0.094	0.52
Thigh	21 d	13.8	13.3	13.1	13.4	13.6	13.2	13.5	0.136	0.23
	35 d	14.5	15.1	14.6	14.5	14.9	14.5	15.1	0.165	0.33
Liver	21 d	2.9[b]	3.2[a]	3.0[b]	2.9[b]	2.9[b]	2.9[b]	2.8[b]	0.014	0.012
	35 d	2.3	2.3	2.4	2.2	2.2	2.2	2.2	0.715	0.10
Proventriculus	21 d	0.48	0.46	0.46	0.46	0.48	0.49	0.45	0.808	0.02
	35 d	0.35	0.36	0.37	0.35	0.35	0.38	0.36	0.779	0.013
Gizzard	21 d	3.13	3.04	2.89	2.82	3.09	3.05	2.95	0.439	0.11
	35 d	2.52	2.38	2.55	2.4	2.45	2.37	2.35	0.566	0.088
Heart	21 d	0.75	0.75	0.71	0.70	0.72	0.71	0.69	0.289	0.022
	35 d	0.91	0.84	0.89	0.91	0.87	0.83	0.83	0.296	0.032
Small intestine	21 d	4.78	4.80	4.94	4.94	4.72	4.98	4.72	0.949	0.214
	35 d	2.94	3.11	3.01	3.07	2.76	3.12	2.9	0.474	0.13
Abdominal fat	21 d	0.22	0.21	0.24	0.17	0.26	0.20	0.23	0.092	0.03
	35 d	0.81	0.70	0.72	0.69	0.70	0.57	0.74	0.707	0.09

[1]Control, control diet, LP10 & LP20, containing 10 and 20% lower CP than control diet, respectively, LP10+EAAs10 & LP20+EAAs10, containing 10% higher EAAs than LP10 & LP20, LP10+EAAs20 & LP20+EAAs20, containing 20% higher EAAs than LP10 & LP20.
[2]Standard error of means. Means with different superscripts within the same row differ significantly (*P* < 0.05)

Table 5. Effects of different treatments on breast meat quality in mixed-Japanese quails at 21 and 35 d of age[1].

variables		Treatments[1]							*P*-value	*SEM*[2]
		Control	LP10	LP20	LP10+ EAAs10	LP20+ EAAs10	LP10+ EAAs20	LP20+ EAAs20		
pH	21 d	6.06	6.03	6.00	6.04	6.01	5.98	6.05	0.858	0.04
	35 d	5.89[b]	6.04[a]	6.05[a]	6.10[a]	6.04[a]	6.01[a]	6.06[a]	0.0001	0.03
Water holding capacity (%)	21 d	73.9	76.2	73.9	68.4	73.1	75.5	76.5	0.403	2.7
	35 d	68.1	69.4	69.5	68.2	69.4	68.1	70.2	0.075	0.56
Cooking loss (%)	21 d	22.2	23.1	22.9	23.9	23.4	24.3	21.9	0.569	0.93
	35 d	24.0	21.1	22.9	21.7	23.7	22.8	21.6	0.348	1.01
Dripping loss (%)	21 d	16.4	16.8	16.7	17.1	16.1	16.7	17.39	0.746	0.52
	35 d	14.7	14.9	17.4	16.0	15.6	14.2	16.3	0.106	0.76

[1]Control, control diet, LP10 & LP20, containing 10 and 20% lower CP than control diet, respectively, LP10+EAAs10 & LP20+EAAs10, containing 10% higher EAAs than LP10 & LP20, LP10+EAAs20 & LP20+EAAs20, containing 20% higher EAAs than LP10 & LP20.
[2]Standard error of means. Means with different superscripts within the same row differ significantly (*P* < 0.05).

At 35 d of age, except for N excretion, other parameters were influenced by different dietary treatments. Quails fed with 17.6% CP diets, with and without EAAs supplementation (LP20, LP20+EAAs10, and LP20+EAAs20), had significantly lower N retention in comparison to those fed control, LP10, and also LP10+EAAs20 diets (*P*<0.01). In both ages, the highest N retention was obtained in quails fed LP10 diets. At 21 and 35 d of age, the lowest N retention was obtained in quails fed LP20+EAAs10 and LP20 diets, respectively.

Table 6. Effects of different treatments on nitrogen retention in mixed-Japanese quails at 21 and 35 d of age.

Variables	Treatments[1]							*P*-value	*SEM*[2]
	Control	LP10	LP20	LP10+ EAAs10	LP20+ EAAs10	LP10+ EAAs20	LP20+ EAAs20		
21 days									
N intake (g/bird/day)	0.68[ab]	0.62[bc]	0.58[c]	0.68[ab]	0.59[c]	0.71[a]	0.60[c]	0.0001	0.018
N excreted (g/bird/day)	0.40[ab]	0.34[c]	0.34[c]	0.37[bc]	0.37[bc]	0.41[a]	0.35[c]	0.0014	0.012
N retention(g/bird/day)	0.29[a]	0.29[a]	0.22[b]	0.30[a]	0.22[b]	0.30[a]	0.24[b]	0.0001	0.012
N retention (% of N intake)	42.89[bc]	46.76[a]	39.41[cd]	45.12[ab]	37.29[d]	41.83[bc]	40.82[cd]	0.0001	1.20
35 days									
N intake (g/bird/day)	0.98[a]	0.91[b]	0.79[cd]	0.84[bc]	0.76[d]	0.92[ab]	0.81[cd]	.0001	0.023
N excreted (g/bird/day)	0.56	0.50	0.51	0.49	0.48	0.52	0.50	0.163	0.019
N retention (g/bird/day)	0.42[a]	0.40[ab]	0.29[d]	0.36[bc]	0.28[d]	0.40[ab]	0.31[cd]	0.0001	0.007
N retention (% of N intake)	42.62[ab]	44.67[a]	38.16[c]	42.50[ab]	38.59[c]	43.71[a]	40.05[bc]	0.0011	1.08

[1]Control, control diet, LP10 & LP20, containing 10 and 20% lower CP than control diet, respectively, LP10+EAAs10 & LP20+EAAs10, containing 10% higher EAAs than LP10 & LP20, LP10+EAAs20 & LP20+EAAs20, containing 20% higher EAAs than LP10 & LP20.
[2]Standard error of means. Means with different superscripts within the same row differ significantly ($P < 0.05$).

Discussion

During the first phase (1-21 d), CP reduction from 25 to 22.5 and 20% significantly decreased daily BWG and increased FCR, whereas adding amino acids to the diet containing 22.5% CP (LP10+EAAs10 and LP10+EAAs20 diets) improved daily BWG and FCR. The results are in line with an earlier study (Kaur *et al*., 2008). Also, Miranda *et al*. (2015) reported decreasing of dietary CP from 24.87 to 22.4% and EAAs supplementation reduced performance in broilers during 1-21 d of age. But, interestingly during the second feeding phase (22-35 d), the daily BWG of chicks fed low CP diets without EAAs supplementation (LP10 and LP20 diets) was more than those fed with the control, LP10+EAAs10 and LP10+EAAs20 diets, even though it was not significant. Because of the compensatory growth, FCR was improved in this period that was in agreement with the results documented by Ali *et al*. (2000) with growing Japanese quails. The degree of compensatory growth response should be related to the severity of amino acid restriction. Another study also showed that feeding broiler chicks by low CP diets supplemented with various combinations of EAAs and nonessential amino acids decreased BWG from 7 to 21 d of age, but not from 21 to 42 d of age (Deschepper and De Groote, 1995). Such *et al*. (2021) reported growth performance of broilers fed with low-protein amino acid-supplemented diet (18.38% CP) was similar to those fed with control diet (20.43% CP) during 11 to 40 d of age.

During the whole rearing period (1-35 d), 10% reduction of CP, without EAAs supplementation, had no significant effect on daily BWG and other performance indices, but 20% CP reduction, without EAAs supplementation, significantly reduced daily BWG, and EAAs supplementation did not improve it. Chrystal *et al*. (2019) attributed these observations to a possible deficiency of amino acids such as serine and glycine in diets with lower crude protein. That small reduction in dietary CP has a little effect on performance has been shown by others (Hernández *et al*., 2012; Miranda *et al*., 2015; Chrystal *et al*., 2019). As dietary levels of CP are further reduced, another EAA becomes limiting and result in reduced performance (Hernández *et al*., 2012; Belloir *et al*., 2017; Chrystal *et al*., 2019). Dean *et al*. (2006) observed that even with supplementation of EAAs, much lower dietary protein may cause a proportion of the EAAs being diverted to form NEAAs because of the lack of nonspecific nitrogen that would be used in this process.

Overall, many researchers noted that decreasing the CP content in poultry diets, and supplementing them with essential amino acids had a positive effect on bird performance (Manoochehri Ardekani *et al*., 2012; Ayasan and Okan, 2014; Belloir *et al*., 2017; Saraiva *et al*., 2020). Several studies also demonstrated that birds fed low CP amino acid supplemented diets didn't perform as well as those fed higher CP diets (Azarnik *et al*., 2010; Nukreaw and Banchasak, 2015; Basavanta Kumar *et al*., 2016). Even so, the information on the optimal protein level for growing quail is still finite. Minoguchi *et al*. (2001) and Alagawany *et al*. (2008) showed that it's possible to decrease the CP level, up to 22% in Japanese quail rations during the growing phase, without any negative effect on growth performance. Wen *et al*. (2017) remarked that with supplementation of crystalline amino acids, it is feasible to formulate the low-protein diets comprising about 22% CP without any adverse effect on growth performance and carcass traits of quails. Santos *et al*. (2016) also demonstrated that reducing dietary CP from 20 to 16% together with EAAs supplementation did not affect laying performance and egg quality in quails. The reason for the difference between these results is not entirely clear, but may be due to the supplementation of the low CP diets with specific amino acids. Diversity in the amount of protein, amino acids fortification, employed ingredients, bird's age, and strain may have contributed to some of the differences in outlined performance (Abudabos and Aljumaah, 2012). In our study, the effect of dietary protein and amino acid levels on livability percentage of quails was not significant that was in line with the other reports (Lima *et al*., 2015; Nukreaw and Banchasak, 2015).

In the present study, there was no effects of dietary treatments on carcass yield at 21 and 35 d of age that coincided with those observed by Aboul-Ela *et al*. (2004) and Alagawany *et al*. (2014). At 21 d of age, the breast yield in the birds fed 22.5% CP diet containing 10% higher EAAs (LP10+EAAs10 diet) was the highest and significantly more than those fed with the control diet. Our findings are in good agreement with Abudabos and Aljumaah (2012), who reported broilers fed low protein diets (19.5% CP) supplemented with EAAs had significantly heavier breasts in comparison to the birds fed with the control diet (21% CP) from 12 to 33 d of age. These authors clarified that one of the reasons could be the greater supply of limiting amino acids, such as lysine and methionine in these diets.

At 35 d of age, there was no significant differences regarding the relative weight of the breast and other carcass parameters between quails fed different dietary treatments. Although, there is a dearth of information on the influence of low-CP diets and various dietary EAAs supplementation levels on carcass characteristics in growing quails, many studies have been published dealing with carcass quality in broiler chickens fed low-CP diets (Miranda *et al*., 2015; Basavanta Kumar *et al*., 2016; Saraiva *et al*., 2020). For instance, Saleh *et al.* (2021) exhibited lowering CP by 2% and EAAs supplementation had no significant effect on the carcass, breast and thigh yield in broilers which was in line with Miranda *et al*. (2015). These authors reported that reducing dietary CP and EAAs supplementation had no significant effect on breast meat yield and abdominal fat in broilers at 42 d of age. On the contrary, Dehghani-Tafti and Jahanian (2016) observed reduced carcass and breast yields in broilers fed low protein diets; however, the proportion of abdominal fat was increased with low protein diets. Belloir *et al*. (2017) reported that reduction of dietary CP and EAAs supplementation did not affect breast meat yield, but, abdominal fat percentage was increased by the reduction of dietary CP content. Similarly, Manoochehri Ardekani *et al.* (2012) reported that reducing dietary protein from 20 to 16% in broiler diets supplemented with synthetic EAAs, significantly increased abdominal fat, but the relative weight of liver, breast, and thigh was not affected by dietary treatments. In our study, lack of a significant effect in FI and FCR between different dietary treatments in total of the rearing period can be partly explained the reason for not affecting abdominal fat. These disagreements among authors might be clarified by many factors such as strain, physiological conditions, age, and production cycle of the birds, diversity in the amount of protein, and amino acid fortification which may influence the efficacy of various low-CP diets supplemented by essential amino acids.

In our study, the relative weight of the liver enhanced in low-CP diets. Wen *et al*. (2017) mentioned that dietary CP reduction from 25.32 to 21.58% and EAAs supplementation increased liver weight in French quails at 42 d of age. It seems, elevated blood ammonia level causes the conversion of ammonia to uric acid in the liver. Hence, this weight enhancement might be attributed to the accommodation to raised ammonia production (Darsi *et al*., 2012). Likewise, the low body weight could be related somewhat to the increasing activity of urea cycle enzymes. It is believed that raising activities of the enzymes involved in uric acid production influences the growth performance (Namroud *et al*., 2008; Darsi *et al*., 2012).

In the present study, dietary treatments had no significant effect on meat quality indices, except for pH. The meat pH in the birds fed with the control diet was significantly lower than the other treatments. Belloir *et al*. (2017) reported reduction of dietary CP from 19 to 16% and EAAs supplementation in broiler diets caused an increase in ultimate pH and a decrease in breast meat dripping loss. Also, Jlali *et al*. (2012) reported reduction of dietary CP from 23 to 17%, and EAAs supplementation increased pH and WHC of breast meat in broilers. Lilly *et al*. (2011) indicated that different dietary densities of EAAs had no significant effect on cooking loss of broilers breast meat at 42 d of age. On the contrary, Gheisari *et al*. (2015) showed that reduction of dietary CP and EAAs supplementation decreased pH of broilers at 42 d of age. Also, no effect on WHC were detected. Also, Tarasewicz *et al*. (2007) reported that feeding quails with a low crude protein diet and providing a similar level of methionine and lysine did not affect pH and cooking loss but increased WHC of breast meat. Different researches have shown that the final pH responds to rearing management, age, pre-slaughter conditions, dietary treatments and especially to variation in methionine, lysine or protein in diet. (Berri *et al*., 2008; Jaturasitha *et al*., 2008; Jlali *et al*., 2012; Conde-Aguilera *et al*., 2016). Duclos *et al.* (2007) and Berri *et al.* (2008) showed that muscle glycogen stores at the time of death, negatively affect the final pH. Moreover, with increase in muscle yield and weight, glycogen levels of breast muscle decrease (Berri *et al.*, 2007). These alterations cause higher pH and WHC of breast meat (Zhang and Barbut, 2005; Albrecht *et al.*, 2019).

According to the results, a 10% reduction in dietary CP decreased N intake and N excreted but had no effect on N retention (g/bird/d). However, a 20% reduction in dietary CP caused a significant reduction in N intake, N excreted and N retention (g/bird/d) at 21 d of age. Similarly, Kerr and Kidd (1999) reported that reduction in dietary CP from 19.4 to 18.2% decreased daily N consumption by broilers with no effect on g N retained/bird/day. Nevertheless, reduction in dietary CP level from 19.4% to 16.7% decreased daily intake of N and g N retained/bird/day. At 35 d of age, except for quails fed LP20+EAAs10 diet, supplementation of the low CP diets had no significant effect on N excretion and N retention percentage compared to the birds fed the control diet. In this respect, Kaur *et al.* (2008) stated that quails fed low-protein diets (20.66% CP) supplemented with EAAs grew slower and retained less N than birds fed with the control diet (25.75% CP). The main event of the improvement of N retention was the decrease in N excretion as shown in Table 6. Reduction of dietary CP up to 10 and 20% decreased N excretion on average 10 and 12.5%, respectively. Similarly, Kaur *et al.* (2008) and Mosaad and Iben (2009) remarked that N retention percentage was significantly higher in growing Japanese quails that fed the high protein diet than those fed the low protein diet. Contrariwise, Dowarah and Sethi (2014) reported that raising dietary protein content from 20 to 24% in Japanese quails significantly decreased N retention at 35 d of age. Kriseldi *et al.* (2018) found that reduction in dietary CP and EAAs supplementation decreased N excretion in broilers in comparison to chicks fed with low dietary CP without EAAs supplementation. In our experiment, these effects were not constant. Eventually, our findings regarding N excretion and N retention well-nigh accommodate to those previously pointed out by other researchers (Dozier *et al.*, 2008; Santos *et al.*, 2016; Ullrich *et al.*, 2018). These authors believe that a suitable strategy to reduce N excretion in poultry farms is reducing the CP level of diet and supplementing with crystalline amino acids.

Conclusion

The findings of this study indicated that a 10% reduction in dietary CP without EAAs supplementation had no adverse effect on the growth performance of Japanese quails during 1 to 35 d of age. Also, at 35 d of age, meat pH of the birds fed with the control diet was significantly lower than the other treatments. EAAs supplementation of low crude protein diets could improve growth performance and reduce environmental pollution, due to reduced N excretion. However, the high cost of some EAAs (e.g., isoleucine and valine) is the main limiting factor in formulating synthetic amino acid-containing diets.

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